PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1038-981 MIS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/00807	03/09/1999	04/09/1998
International Patent Classification (IPC) or national classification and IPC C12N15/37		
Applicant CONNAUGHT LABORATORIES LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 40 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/03/2000	09.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Lanzrein, M Telephone No. +49 89 2399 7358

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/00807

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-25 as originally filed

Claims, No.:

1-13 as originally filed

Drawings, sheets:

1/8-8/8 as originally filed

Sequence listing part of the description, pages:

1-4, filed with the letter of 10.01.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☒ furnished subsequently to this Authority in written form.
   ☒ furnished subsequently to this Authority in computer readable form.
   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 2, 3, 5-10
	No:	Claims 1, 4, 11-13

Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-13
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: WO 92 16636 A (IMMUNOLOGY LTD) 1 October 1992 (1992-10-01)

D2: MUENGER K. ET AL.: 'COMPLEX FORMATION OF HUMAN PAPILLOMAVIRUS E7 PROTEINS WITH THE RETINOBLASTOMA TUMOR SUPPRESSOR GENE PRODUCT' EMBO JOURNAL, vol. 8, no. 13, 20 December 1989 (1989-12-20), pages 4099-4105.

D3: WO 96 00583 A (MERCK & CO INC ;DONNELLY JOHN J (US); LIU MARGARET A (US); MARTINE) 11 January 1996 (1996-01-11)

D4: SUNDARAM P. ET AL.: 'Intracutaneous vaccination of rabbits with the E6 gene of cottontail rabbit papillomavirus provides partial protection against virus challenge. ' VACCINE, vol. 16, no. 6, April 1998 (1998-04), pages 613-623.

D5: RESSING M. E. ET AL.: 'HUMAN CTL EPITOPES ENCODED BY HUMAN PAPILLOMAVIRUS TYPE 16 E6 AND E7 IDENTIFIED THROUGH IN VIVO AND IN VITRO IMMUNOGENICITY STUDIES OF HLA-A0201-BINDING PEPTIDES' JOURNAL OF IMMUNOLOGY, vol. 154, 1 June 1995 (1995-06-01), pages 5934-5943.

Re Item IV
Lack of unity of invention

The international preliminary examining Authority is of the opinion that the present application lacks unity within the meaning of Art. 34(3) and Rule 13.1 PCT.
It will be considered that the following separate alleged inventions are not so linked as to form a single general inventive concept:

1) Claims 5, 6 (completely), claims 1-4, 11-13 (partially): A vector comprising a sequence encoding a HPV16 E7 protein which has a reduced oncogenic potential due to deletion of amino acids 21-26

2) Claims 7-10 (completely), claims 1-4, 11-13 (partially): A vector comprising a sequence encoding the immunogenic epitopes of E7 (amino acids 11-20, 49-57, 82-90, 86-93) and of E6 (amino acids 29-38).

The general inventive concept underlying the above mentioned inventions 1) and 2), can be seen in the decreased oncogenicity of E7. The oncogenic potential was reduced by either deleting the RB-binding sequence (invention 1) or by by assembly of the immunogenic epitopes of E7 and E6 without the intervening sequences (invention 2). However, said general inventive concept is not novel in view of D1.
The need for reduction of the oncogenic potential of E7 used for immunotherapy or vaccination was recognized in D1. The problem was solved by the provision of vaccinia virus vectors expressing fusion proteins of E6 and E7 from HPV 16 and HPV 18. The recombinant vectors are proposed for use as immunotherapeutics or vaccines for conditions related to HPV infection, such as cervical cancer (p. 4, l. 19-23). The oncogenic potential of HPV 16 E7 was reduced by replacement of cys 24 and glu 26 (cys 27 and glu 29 in HPV 18) with glycine (p. 12, l. 12-18; p. 17, l. 5-11; p. 25, l. 28 - p. 26, l. 14; Fig. 2). The two mutated residues are involved in RB-binding and the block of this interaction apparently reduces oncogenicity.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application concerns vectors expressing E6 and/or E7 protein of human papilloma virus (HPV). The oncogenic potential of E7 is reduced by a deletion (amino acids 21-26) or by assembly of the immunogenic epitopes of E7 and E6 without the intervening sequences. The constructs are to be used for DNA vaccination against HPV-associated cervical cancers. The applicants demonstrate experimentally that both constructs induce protective antitumor immunity in mice challenged with C3 tumour cells (examples 5-10).

2. Claims 1, 4, 11-13 lack novelty according to Art. 33 (2) PCT in view of D1.
D1 describes vaccinia virus vectors expressing fusion proteins of E6 and E7 from HPV 16 and HPV 18. The recombinant vector is proposed for use as an immunotherapeutic or vaccine for conditions related to HPV infection, such as cervical cancer (p. 4, l. 19-23). The oncogenic potential of HPV 16 E7 was reduced by replacement of cys 24 and glu 26 (cys 27 and glu 29 in HPV 18) with glycine (p. 12, l. 12-18; p. 17, l. 5-11; p. 25, l. 28 - p. 26, l. 14; Fig. 2). Hence, the residues involved in binding of RB were mutated, thereby abolishing the immortalizing potential of E7.

   Claims 2, 3, 5-10 are found to be novel over the cited prior art.

3. Claims 2, 3, 5-10 lack inventive step according to Art. 33 (3) PCT.
For evaluation of inventive step, the two inventions identified in the previous communication (invitation to restrict or pay additional fees) are analysed separately.

3.1 Invention 1): Claims 5, 6 (completely), claims 2, 3 (partially): A vector comprising a sequence encoding a HPV16 E7 protein which has a reduced oncogenic potential

due to deletion of amino acids 21-26.

The closest prior art document is D1. The vaccinia vector of D1 expressing E6/E7 has reduced oncogenic potential due to mutation of the RB-binding site (p. 12, l. 12-18; p. 17, l. 5-11; p. 25, l. 28 - p. 26, l. 14; Fig. 2). The vector is to be used as an immunotherapeutic or vaccine for conditions related to HPV infection, such as cervical cancer (p. 4, l. 19-23). The difference to the vector of the present application is thus: i) another mutation in the RB binding site (i.e. deletion of amino acids 21-26) and ii) the use of a plasmid vector with CMV promotor instead of vaccinia.
The technical problem underlying the present alleged invention can therefore be seen in the provision of further vaccines against HPV-associated cervical cancer.
The solution proposed cannot be considered involving an inventive step (Article 33(3) PCT) for the following reasons:

The closest prior art document D1 used vaccinia virus vectors for vaccination. D3 and D4 both disclose DNA vaccines for papilloma virus. Thus, the skilled person was well aware of the successful use of DNA vaccines for HPV E6 (D4) or E7 (D3). There was an existing need (mentioned in D1 p. 5, lines 10-15; or D4 p. 622) to improve the vaccines in terms of the oncogenic potential. Further, the skilled person knew from D1 that abolishment of RB-binding did reduce the oncogenic potential of HPV E7. D2 discloses that deletion of amino acid residues 21-24 of HPV16 E7 abolished binding of RB and mutation of E26 severely impaired RB-binding (p. 4103, right column, 2. paragraph). This showed clearly that the RB-binding site consisted of amino acids 21-26. It can therefore not be considered inventive to delete amino acids 21-26 for the purpose of reducing oncogenic potential. This is just an arbitrary selection of another possible mutation. It cannot be seen at present whether any unexpected technical effect is associated with this.

3.2 Invention 2): Claims 7-10 (completely), claims 2, 3 (partially): A vector comprising a sequence encoding the immunogenic epitopes of E7 (amino acids 11-20, 49-57, 82-90, 86-93) and of E6 (amino acids 29-38).
The feature which distinguishes alleged invention 2) from alleged invention 1) is

the approach to reduce oncogenic potential by expressing immunogenic epitopes of HPV E6/E7.
The epitopes of E6 and E7 were known from D5. It is stated that the epitopes "could be used in vaccines for the prevention and treatment of cervical carcinoma" (abstract). It is shown that immunity against multiple epitopes can be induced by administration of a mixture of the four immunogenic HPV peptides E6.29-37, E7.11-20, E7.82-90, E7.86-93 (p. 5937, left hand column, last paragraph; Table II). Furthermore, it is stated in D4 that *"several E6-expressing constructs can be generated to express individually, complementary portions of the E6 protein. By combining these in a single multivalent E6 vaccine, it should be possible to induce the full spectrum of E6 antigenicity while, simultaneously, eliminating any pathogenicity associated with full-length E6 proteins."* Thus, D4 teaches to combine the individual epitopes in a single vaccine. D4 also teaches the use of HPV E6 DNA vaccines. DNA vaccines using the E7 coding sequence were also known from D3.
We hold the view that the skilled person would, in order to provide an alternative vaccine for HPV, use the well characterized epitopes of HPV E6/E7. In view of D4 and D5, it was obvious to use multiple epitopes in combination.

4. Claims 12 and 13 concern methods of treatment of the human or animal body. For the assessment of said claims on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99/18995	22.04.1999	09.10.1998	09.10.1997

This document discloses DNA vaccines of HPV16 E7 derived immunogenic peptides.

Re Item VIII
Certain observations on the international application

1. Claims 1, 4 and appending claims 2, 3, 11-13 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. Furthermore, the terms used to specify the result to be achieved are unclear. It is not clear to the skilled person what is meant by a "non-toxic T-cell epitope" or a "sequence detoxified to prevent oncogene replication".

2. The arbitrary terms designating plasmids employed in claims 3, 6, 10 are not generally accepted in the field in question, contrary to the requirements specified in the PCT Guidelines II-4.15.